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Review

Phenotypic heterogeneity in fungi: Importance and methodology



Sarah K. HEWITT, David S. FOSTER, Paul S. DYER, Simon V. AVERY*

School of Life Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

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ABSTRACT

Phenotypic heterogeneity describes the variation that exists between individual cells, spores or other biological entities within genetically-uniform populations of fungi or other organisms. Studies over the last 10–15 years have successfully used laboratory- and modelling-based approaches to demonstrate the prevalence of phenotypic heterogeneity and characterise the molecular bases of the phenomenon (primarily centred around heterogeneous gene expression). In contrast to progress in these areas, the relevance of phenotypic heterogeneity for the competitive success of organisms in different natural scenarios, although widely speculated upon, has only recently begun to be investigated. This review addresses this latter question as tackled in recent studies with yeasts and filamentous fungi. We concentrate on the relevance to fungal activities such as survival against environmental stressors, pathogenesis, and spoilage. We also discuss methodologies for interrogating phenotypic heterogeneity in fungi. The emerging prevalence and apparent importance of fungal phenotypic heterogeneity provides a timely reminder that certain, potentially core aspects of fungal biology still remain widely under-explored.

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1. Introduction

In the context of microbiology, ‘phenotypic heterogeneity’ refers to the phenomenon whereby individual cells within clonally-derived populations, that have a uniform genetic background, can nevertheless display differences in phenotype (i.e., heterogeneity). This phenomenon (also termed ‘non-genotypic heterogeneity’) is likely to be observable in almost any phenotype. One classic example is evident when clonally-derived microbial cells are exposed to harmful stressors (Sumner *et al.*, 2003; Levy *et al.*, 2012; Holland *et al.*, 2014; Guyot *et al.*, 2015). In this situation it is frequently

observed that not all of the cells of a population will lose viability simultaneously. Instead, a minority of cells often survive at levels of exposure that kill most of the sibling cells, despite all of the cells being genetically uniform. In the case of fungi, phenotypic heterogeneity may encompass variation seen between genetically-uniform populations of single cells, such as those formed by ascomycete and basidiomycete yeasts, between genetically identical mitospores, and between genetically uniform hyphal compartments of filamentous fungi. Several mechanisms underlying heterogeneity have been described, which are largely manifested via differential gene expression (gene expression noise). That is, if one or

* Corresponding author.

E-mail address: Simon.Avery@nottingham.ac.uk (S. V. Avery).
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more genes influencing a particular phenotype are expressed at a different level in different cells or spores, heterogeneity in the phenotype is likely to result. Therefore, heterogeneous phenotypes are usually traceable to heterogeneity in levels of particular transcripts or in their (post-)translational regulation. The molecular bases for heterogeneity have been reviewed in depth elsewhere (Avery, 2006; Munsy et al., 2012; Coulon et al., 2013; Symmons and Raj, 2016).

Phenotypic heterogeneity is potentially of great significance in fungal biology and ecology. For example, it may result in the survival of a subset of cells or hyphae which may then go on to mount adaptive responses and allow re-establishment of populations, critical for the survival of species (Avery, 2006). Furthermore, given that the degree of phenotypic heterogeneity can vary between different strains of the same species, it can provide an extra, often not appreciated, level of variation conferring an adaptive advantage upon which natural selection can act (Blake et al., 2006; Yvert et al., 2013; Holland et al., 2014).

Phenotypic heterogeneity is also potentially of great applied significance. In addition to survival in response to environmental stress, the phenomenon might also be important in terms of virulence whereby certain cells within a population might display enhanced pathogenicity and/or resistance to antifungal drugs (LaFleur et al., 2006; Halliwell et al., 2012; Pierce and Kumamoto, 2012; Bezerra et al., 2013). Similarly, with respect to food spoilage certain cells or spores might be able to survive treatment by preservatives that otherwise kill all other members of the population (Steels

et al., 2000; Stratford et al., 2013). Finally, there might also be biotechnological applications if certain cells within populations produce higher levels of desirable metabolites or proteins (Papagianni, 2004; Krijgsheld et al., 2013; Xiao et al., 2016).

2. Methods for determining phenotypic heterogeneity in fungi

The ability to examine single cells microscopically and to culture them discretely as single colonies has been available for nearly as long as the field of microbiology has existed. It is only with the emergence of phenotypic heterogeneity as a major field of study over the last 10–15 years that a range of new methods have been developed to examine phenotypic variability between single yeast and other fungal cells. Phenotypic variability may be present in a population as part of a normal distribution, skewed distribution or biphasic distribution, reflective of the underlying mechanism or evolutionary strategy. Therefore, examining the shape as well as the extent of the phenotype's distribution can help resolve the source and role of phenotypic heterogeneity in a population. Colony forming ability provides a classic binary assessment of single cell viability (Table 1). A quantitative measure of heterogeneity in response to a drug or stressor can be achieved by comparing the ability of single cells to form colonies over a range of doses (Fig. 1). The gradient of the dose–response curve depicts the extent of population stress-response heterogeneity, revealing

Table 1 – Methods for quantifying phenotypic heterogeneity in fungal populations.

Approach	Method	References
Dose-response analysis	% Colony forming units (CFUs) in microtiter wells	Steels et al. (2000), Stratford et al. (2013), De Brucker et al. (2016)
	% CFUs on agar dishes	Sumner et al. (2003), Stratford et al. (2013), Holland et al. (2014), De Brucker et al. (2016)
Flow cytometry	Gene expression reporters	Blake et al. (2006), de Bekker et al. (2011b), Levy et al. (2012), Liu et al. (2015)
	Metabolic staining	Kell et al. (1991), Lloyd et al. (1996), Davey et al. (2004), Noda (2008), Guyot et al. (2015)
	Live/dead staining	Wenisch et al. (1997), Attfield et al. (2001), Guyot et al. (2015)
Single cell, hyphal and colony imaging	Intracellular cytoplasmic-pH measurement	Weigert et al. (2009), Stratford et al. (2014)
	Single cell X-ray	Crawford et al. (2016)
	High throughput single cell microscopy	Levy et al. (2012), Bauer et al. (2015)
	Microfluidics	Fehrmann et al. (2013), Nobs and Maerkl (2014), Hansen et al. (2015), Zhu et al. (2015), Lee et al. (2016)
	Macrocolony size/growth rate variation	Stratford et al. (2014)
	Microcolony size/growth rate size variation	Levy et al. (2012), Ziv et al. (2013)
	Filamentous fungal macrocolony imaging	Bleichrodt et al. (2012), Vinck et al., (2005, 2011)
	Membrane-fluidity probing	Guyot et al. (2015)
	Fluorescence markers of single-cell growth	Di Talia et al. (2007), Carlquist et al. (2012)
	BONCAT-FISH ^a	Hatzenpichler et al. (2014)
Mass spectrometry	Nonlinear spectral microscopy (NLSM)	Knaus et al. (2013)
	Single cell ICP-MS	Groombridge et al. (2013), Wang et al. (2015)
	Microarrays for mass spectrometry (MAMS) platform	Ibanez et al. (2013)
Other	NanoSIMS ^a	Zimmermann et al. (2015)
	Single-cell RNA-seq ^a	Tang et al. (2010), Fan et al. (2015)
	Single-hypha transcriptomics	De Bekker et al. (2011a)
	Zonal secretomics in fungal mycelium	Krijgsheld et al. (2013)

^a Method developed in other cell systems, but with potential for application in fungi.

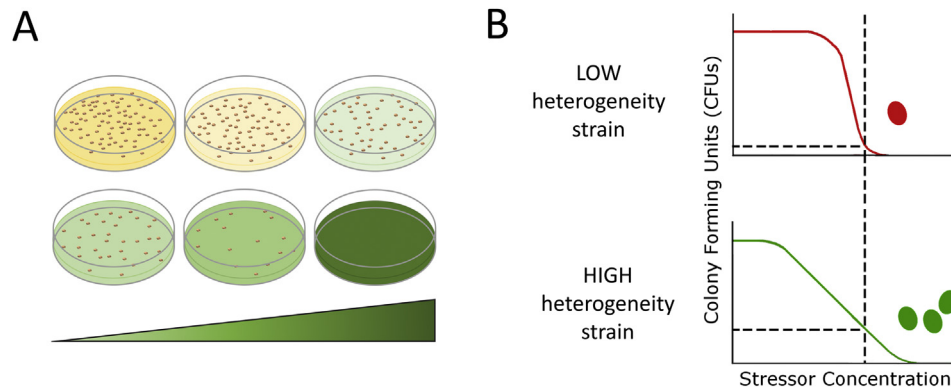


Fig. 1 – Dose response analysis for quantification of heterogeneity. (A) Decline in colony forming units (CFUs) is determined across a range of increasing stressor doses. (B) The slopes of the resultant dose–response curves reflect the extent of heterogeneity in resistance to the stressor, with a shallow slope representing high heterogeneity. In the example shown, the indicated high dose (vertical dotted line) yields a larger proportion of surviving cells in the high heterogeneity population, despite this population having a lower mean-resistance (IC_{50} ; not marked).

the presence of subpopulations or rare persisters in a population (Sumner et al., 2003; Bishop et al., 2007; Stratford et al., 2013; Holland et al., 2014). Fluorescent probes are valuable, alternative indicators of single cell phenotypes. Flow cytometric analysis of cell fluorescence is tailor-made for single cell measurements and can be used to examine heterogeneity of spore, germling or yeast populations according to gene expression (Blake et al., 2006; Levy et al., 2012; Liu et al., 2015), cell viability (Wenisch et al., 1997; Attfield et al., 2001; Guyot et al., 2015) or cell vitality; the latter application may make use of dyes sensitive to membrane potential such as Rhodamine 123 and DiBAC₄(3) (Davey et al., 2004; Guyot et al., 2015) or calibrated measurements of intracellular pH which reflect proton extrusion rate as an indicator of metabolic function (Weigert et al., 2009; Stratford et al., 2014). Crucially, when assessing phenotypic heterogeneity using flow cytometry, care should be taken to eliminate the potentially confounding factors of individual cell size and the presence of cell aggregates (e.g., cell doublets versus budding cells) by using appropriate gating and/or mathematical corrections (Wersto et al., 2001; Knijnenburg et al., 2011). Probes such as Phloxine B that report on metabolism-dependent membrane transporter activity also provide a useful measure of vitality (Noda, 2008) (Fig. 2). More recent developments have included flow cytometric measurement of the uptake of stressors like metals into individual cells (Crawford et al., 2016); differential uptake of drugs or stressors in different cells is one likely cause of heterogeneous responses or adaptation. Related to this, mass spectrometry-based methods employ single cell ICP-MS (Groombridge et al., 2013; Wang et al., 2015) and microarray for mass spectrometry (MAMS) platforms (Ibanez et al., 2013) for elemental and metabolome analysis in single cells, respectively. Furthermore, techniques such as nanometer-scale secondary ion mass spectrometry (NanoSIMS) (Zimmermann et al., 2015) have been used to quantify phenotypic heterogeneity in N_2 and CO_2 fixation activity in bacterial cells and may have future applications in fungi. Another technique with potential application to fungi is fluorescence microscopy-based bioorthogonal noncanonical amino acid tagging (BONCAT) (Dieterich et al., 2006). BONCAT enables the

quantification and tracking of protein synthesis in single cells. It can be used in combination with other single-cell methods like rRNA-targeted fluorescence *in situ* hybridisation (FISH) for concurrent taxonomic identification in environmental microbial samples (Hatzenpichler et al., 2014), potentially enabling *in situ* snapshots of microbial phenotypic heterogeneity. Single-cell RNA-seq provides phenotypic data at its primary source, gene expression. Single-cell RT-PCR has been widely used while more genome-wide technologies have been, and continue to be, developed including the Fluidigm® and molecular-indexing platforms (Tang et al., 2010; Fan et al., 2015). As the cost and technical difficulty of these approaches decreases, their application will expand beyond current limited contexts and may

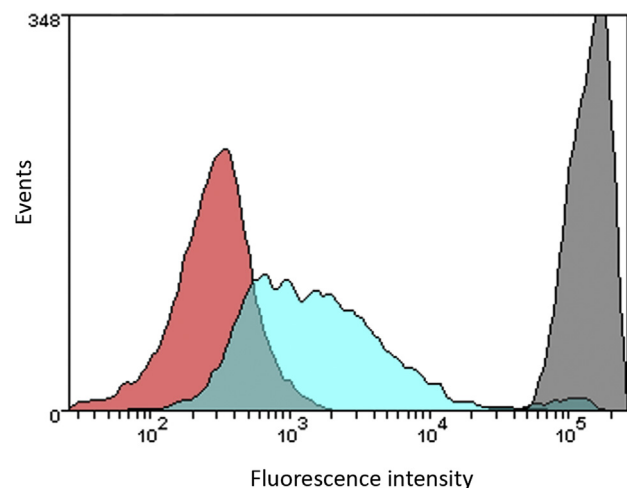


Fig. 2 – Vitality analysis of single yeast cells. Flow cytometric analysis of wild type *S. cerevisiae* (red), a high-heterogeneity *vma3Δ* mutant (Bishop et al., 2007) (blue), and heat-killed cells (grey), after staining with vitality probe Phloxine B (Noda, 2008). Increasing fluorescence indicates decreasing vitality, with the width of the peaks reflecting population heterogeneity. Unpublished data: S.K. Hewitt, P.S. Dyer, S.V. Avery.

provide new dimensions in the analysis of fungal phenotypic heterogeneity.

Growth rate heterogeneity

Several of the above approaches may help to infer the heterogeneity of physiological status within a cell population by relaying information concerning variation in substrates or metabolites present in single cells. However, in most cases these measurements relate to specific metabolic pathways or functions that might not necessarily reflect global physiological status. The latter is important for addressing many questions relevant to phenotypic heterogeneity and there is probably no better measure of the global physiological status of a cell than its growth rate (Dusny and Schmid, 2015). To assess growth rates of single yeast cells in high-throughput, the formation of microscopic colonies on agar has been monitored over time, in an automated manner (Levy et al., 2012). The reader is referred to Weaver et al. (2014) for a focused review of high throughput single-cell microtechnologies. Heterogeneity of micro-colony growth rates was increased in a number of yeast deletion mutants, implying a role for the deleted functions in buffering variation of single cell (micro-colony) growth rate (Levy et al., 2012). A recent adaptation of this approach involved analysis of yeast microcolony formation in broth media, which allowed purification of slow and fast-dividing cells by FACS (van Dijk et al., 2015). Variation in size of macroscopic (visible) colonies may serve as a downstream indicator of variation in the initial division-rates of cells on solid medium. It should be noted that the further from those initial cell-divisions that the measurement is taken, the more blunted it may become due to subsequent divergences in growth rates of individual cells within each growing macrocolony. Increased macrocolony size heterogeneity was observed in *Zygosaccharomyces bailii* under weak acid stress, due to a slow-growing, resistant subpopulation (Stratford et al., 2014). A more optimal level of fitness, via stress resistance for example, may develop over time by specialisation of individual cells within a colony and cooperation with neighbouring cells, such as by metabolite exchange or toxicant removal (Ackermann, 2015; Campbell et al., 2015, 2016).

Advances in microscopy and microfluidic technologies have enabled improved direct monitoring and modelling of single cell growth-rate phenotypes. A number of single-cell morphological characteristics can be analysed via principal component analyses (PCA) to reflect the “phenotypic potential” of a population, allowing comparison of the level of cell–cell variation between yeast strains (Yvert et al., 2013; Bauer et al., 2015). The application of other high-content imaging and statistical tools to single-cell studies have been discussed elsewhere (Geiler-Samerotte et al., 2013). Time-lapse microscopy with single *S. cerevisiae* cells expressing a fluorescent reporter of actin-gene expression was found to provide a reliable indicator of cell size and single-cell growth rate (di Talia et al., 2007). A reporter of ribosomal-protein gene expression has been used elsewhere for a similar purpose (Carlquist et al., 2012). These approaches to trace single-cell growth rate require a genetically manipulatable host. Capture or adherence of single cells within microfluidic chips allows for long-term monitoring of single cell division

and tracking over a number of generations. Most devices are designed for replicative life-span measurement, but could be adapted for comparison of single cell growth rate under different conditions or between different strains (Fehrmann et al., 2013; Nobs and Maerkl, 2014; Hansen et al., 2015; Chen et al., 2016). As above, the most desirable methods for heterogeneity applications would provide short-term snapshots of instantaneous growth rate, not subject to averaging effects of a growth-rate measurement that spans generations. Electrical impedance spectroscopy with single cells has been achieved by incorporation of electrodes into microfluidic devices, thereby allowing the non-invasive monitoring of cell length and nuclear division of trapped *Schizosaccharomyces pombe* cells (Zhu et al., 2015). However, *Saccharomyces cerevisiae* is not so easily trapped and oriented, causing inconsistent measurements in this species (Zhu et al., 2014).

Heterogeneity in filamentous fungi

Filamentous fungi pose a more complex model for investigating phenotypic heterogeneity. Their largely syncytial cytoplasm creates a more genetically intricate environment, which can be partially compartmentalised by septa in ascomycete and basidiomycete fungi. This means that gene expression by multiple nuclei can contribute to the phenotype of a given hyphal compartment, suggesting that some averaging of any gene expression noise might occur and result in reduced heterogeneity. However, many filamentous fungi only contain 2–4 nuclei per compartment (Kaminskyj and Hamer, 1998; Shen et al., 2014). Furthermore, studies on model species suggest that nuclear division only occurs at apical tips and is largely synchronous (Kaminskyj and Hamer, 1998; Momany, 2001; Gladfelter, 2006). Given that apical tips will be at varying stages of development (hence with nuclei at different stages of the cell cycle) there is a further possibility for non-genotypic heterogeneity. Indeed there is some evidence of this phenomenon in ascomycete fungi, discussed further below (de Bekker et al., 2011a, 2011b; Bleichrodt et al., 2012, 2013, 2015b).

There is scope to measure phenotypic heterogeneity at least at three different levels in filamentous fungi: variation between individual spores (e.g., during spore formation, quiescence or germination), variation between individual hyphae of a single mycelium (Wösten et al., 2013), or variation in a mean phenotype between different, isogenic mycelia (Fig. 3). In principle, variation may arise at any of these levels and in any phenotype, including rate of hyphal growth or resistance to environmental stressors and antifungals. Although several of the methods described above are applicable to fungi generally, there remains a scarcity of dedicated approaches for investigating heterogeneity in the filamentous growth form. This is set to change, with some of the newest developments encompassed in the next section, below.

3. Role of phenotypic heterogeneity in the biology and performance of fungi

The prevalence of phenotypic heterogeneity in fungi and other microorganisms is now well established, across a range of

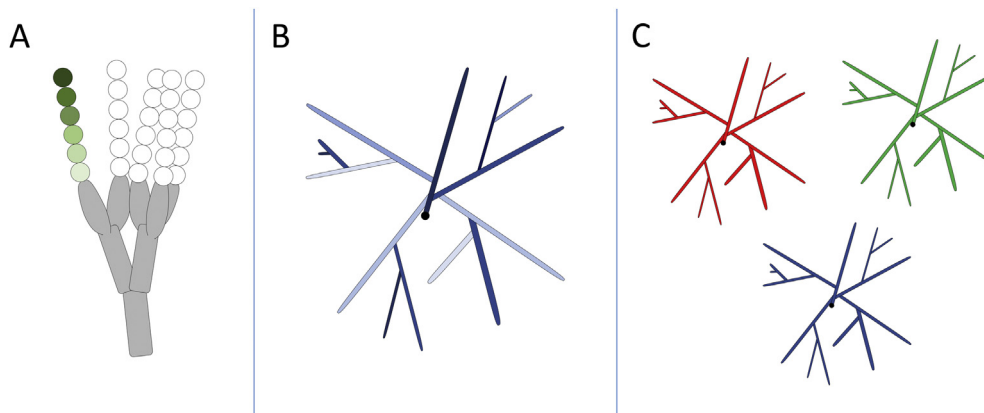


Fig. 3 – Different forms of phenotypic heterogeneity in a filamentous fungus. (A) Inter-spore variation. (B) Variation between individual hyphae of a single mycelium. (C) Variation in average phenotype between isogenic mycelia.

phenotypes. What is far less widely characterised is the impact of such heterogeneity, beneficial or otherwise, on the competitiveness or general biology of fungi. Phenotypic heterogeneity and/or the presence of rare survivors has been demonstrated to increase fitness or survival of fungal populations exposed to stressors in the laboratory (Blake et al., 2006; Smith et al., 2007; Bishop et al., 2007; Holland et al., 2014; Liu et al., 2015). Several of these studies have compared populations matched for mean phenotype (e.g., IC_{50}) but with differing heterogeneities. The benefits of high cell–cell variability have also been demonstrated computationally: modelling and simulations can function both as complementary (Blake et al., 2006) and stand-alone (Hellweger et al., 2014) methods for validating or predicting the influence of phenotypic heterogeneity on fungal populations, particularly in fluctuating environments (Thattai and van Oudenaarden, 2004; Acar et al., 2008; Gaal et al., 2010). There is evidence that the extent or dynamics of phenotypic heterogeneity in yeast populations may become tuned to their particular environment (Acar et al., 2008; Yvert et al., 2013). Recently it was established that increases in the heterogeneity (and epigenetic inheritance) of yeast-cell division times could result in increased population growth rate, i.e., increased fitness (Cerulus et al., 2016).

Environmental stress

Studies have begun to assess the potential roles of phenotypic heterogeneity in the success of fungi in the contexts of fungal ecology (Yvert et al., 2013; Holland et al., 2014; Guyot et al., 2015), pathogenesis (Halliwell et al., 2012; Pierce and Kumamoto, 2012) and food spoilage (Steels et al., 2000; Stratford et al., 2013, 2014). From an ecological perspective, populations with high cell–cell variability may be better equipped to deal with anthropological environmental stresses such as industrial pollutants. Wild yeast populations isolated from heavy metal or sulphur dioxide-contaminated environments showed a higher level of heterogeneity (in resistance to the relevant toxicants) compared to isolates of the same species from unpolluted control sites, and this heterogeneity appeared to have been subject to stronger evolutionary selection than the trait of population-mean resistance (Holland

et al., 2014). A high level of heterogeneity has also been observed in the response to heat shock of *S. cerevisiae*, with individual-cell thermal resistance being negatively correlated with growth rate (Levy et al., 2012). Slower growing cells tended to have a higher replicative age, a correlation which may form the basis for an age-dependent bet-hedging strategy that benefits yeast cell populations, at least under low frequency, high intensity heat shock (Hellweger et al., 2014). In addition, slower growing cells have a higher freeze-thaw stress resistance, whereby cells exhibit a trade-off between membrane robustness (ability to withstand freeze-thaw cycles) and growth rate (Carlquist et al., 2012). Interestingly, Guyot et al. (2015) identified subpopulations within *S. cerevisiae* cultures in which thermal damage was reversible and these cells were able to seed recovery of entire populations, providing an example of the advantages of a heterogeneous population.

Evidence for phenotypic heterogeneity in filamentous fungi

Recent work supports the prevalence of non-genotypic variation also in filamentous fungi. Nonlinear spectral microscopy (NLSM) non-invasively maps the autofluorescence emission spectra of fluorophores in biological samples (Zipfel et al., 2003). By measuring the fluorophores NAD(P)H, flavin adenine dinucleotide (FAD) and melanin, NLSM has been successfully applied to mushrooms of *Agaricus bisporus* to demonstrate heterogeneous metabolism and pigment formation within zones throughout individual mushrooms, and even between neighbouring hyphae (Knaus et al., 2013). Transcript and protein levels and protein secretion have been reported to vary both between adjacent hyphae, spatially across colonies and between micro-colonies of *Aspergillus* spp. (Wösten et al., 1991; de Bekker et al., 2011a, 2011b; Vinck et al., 2005, 2011; Krijgheld et al., 2013). Expression of GFP from different gene promoters produced heterogeneous levels of fluorescence between adjacent hyphae of *Aspergillus oryzae* (Bleichrodt et al., 2012). This heterogeneity was suppressed by deletion of the *Aohex1* gene, which encodes a Woronin body structural protein. Evidence of transient septal plugging by Woronin bodies in living hyphal segments was also reported (Bleichrodt et al.,

2012). This supported a role for Woronin bodies in functions other than response to membrane damage, such as possibly restricting both the streaming and even distribution of organelles, proteins, RNA and nutrients through the cytoplasm. Such an action may create a more heterogeneous, multicellular structure in healthy mycelia (Bleichrodt et al., 2015a). It is reasoned that resultant variations in gene expression between individual hyphae or hyphal compartments may dictate heterogeneous phenotypes within discrete sections of mycelia. Novel microfluidics-based tools for monitoring long-term growth of individual hyphae could help to tease out such consequences of hyphal heterogeneity (Lee et al., 2016).

Pathogenic and food-spoilage fungi

From a clinical point of view, high heterogeneity in the cell population of a fungal pathogen may allow for persistence in the face of antifungal drugs or host defences. Halliwell et al. (2012) showed extremely high heterogeneity in the expression of the virulence-associated adhesin Epa1, of *Candida glabrata*. The individual-cell Epa1 expression level determined the level of adherence to epithelial cells. *Candida glabrata* undergoes phenotype switching in response to copper and other environmental stresses, and switching can occur at infection sites (Brockert et al., 2003; Srikantha et al., 2005). There are a number of examples of phenotypic switching in other fungi, which can contribute to virulence (Jain and Fries, 2009). The role of phenotypic heterogeneity in producing antibiotic-resistant persister cells is a phenomenon that is well reported in bacteria and which continues to gain research traction (Gefen and Balaban, 2009; El-Halfawy and Valvano, 2015). Regarding pathogenic yeast, persistent individuals of *Candida albicans* are thought to contribute to the elevated antibiotic resistance seen during biofilm formation (LaFleur et al., 2006; De Brucker et al., 2016). This, like some other aspects of phenotypic versatility in fungal pathogens (e.g., adaptation to different host niches), can reflect adaptive responses to different (micro)environments which does not strictly fall within the scope of this review.

The chaperone Hsp90 may alter the virulence potential of fungal pathogens, by modulating in different ways the translation of genotype (and genotypic variation) to phenotype (and phenotypic variation) (reviewed by Veri and Cowen, 2014). Phenotypic diversity in fungi can also be promoted by prion-dependent errors in mRNA translation or transcriptional repression (Halfmann et al., 2012). Heterogeneity in the expression of the *C. albicans* transcription factor, Efg1, is proposed to enable rapid responses to host immune status, which is potentially crucial in the balance between commensal and pathogenic lifestyles of a host's resident *C. albicans* population (Pierce and Kumamoto, 2012). Furthermore, proteome instability caused by leaky amino acid incorporation during protein synthesis of *C. albicans*, has the potential to promote phenotypic diversity which impacts drug resistance and host immune cell responses (Bezerra et al., 2013).

Heterogeneity in resistance to stress presents a major problem in the food industry, because it increases the likelihood that resistant individuals will thrive in foodstuffs usually considered inhospitable to microorganisms, or will

survive preservative procedures. The food spoilage yeast *Z. bailii* exhibits high resistance to weak-acid preservatives, which is conferred by a small proportion of the population which survives high-acid conditions (Steels et al., 2000; Stratford et al., 2013). Heterogeneity in the cytosolic pH of individual cells is one factor that determines single-cell weak acid tolerance of yeast (Fernandez-Nino et al., 2015). Rare survivors of weak acid preservative were more common in stationary phase than exponential phase cultures (Stratford et al., 2014), suggesting one basis for common observations of population-averaged stress resistance in stationary phase organisms. The evidence to date from such studies reinforces the notion that whereas phenotypic heterogeneity is beneficial from a fungal perspective in that it can enhance the fitness of populations in changing environments, it may have detrimental impacts on our battle to control undesirable pathogenic and spoilage fungi. Focused work is now needed to test these ideas rigorously.

4. Concluding remarks

Despite recent progress, the phenotypic consequences of heterogeneity in ecological, clinical and industrial contexts are still largely under-tested and under-studied, especially in filamentous fungi and basal fungal species such as single cell chytridiomycota and the zygomycota. These are key challenges now, with potentially major implications for understanding how fungi interact successfully with their environments. It seems likely that phenotypic heterogeneity enhances the competitiveness of fungi in their diverse lifestyles, including those both beneficial and detrimental to us. The potential exploitation of phenotypic heterogeneity for improving fungal processes in biotechnology is a further area that has yet to be explored. Xiao et al. (2016) recently developed a procedure for continuously selecting high-performing, non-genotypic variants within bacterial cell populations, to improve yield from production processes by up to three-fold. Such examples could pave the way for similar applications in fungal biotechnology. Indeed, in the coming years we anticipate that phenotypic heterogeneity will become increasingly recognised as a key fungal trait, modulating diverse, fundamental aspects of fungal activity.

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